# マウスのMycobacterium avium腸管感染


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# *Mycobacterium avium* Infection through the Alimentary Tract in Mice

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ABSTRACT. Intestinal infection by *Mycobacterium avium* was investigated in C57BL/6 and BALB/c mouse strains. Single intragastric administration of a massive dose ($10^8$) or multiple administration of a lower dose ($10^7$, 10 times) established infection principally in the mesenteric lymph-node (MLN); a continuous or intermittent fecal excretion of the bacilli was detected by 6–8 weeks after the administration. Based on three criteria—isolation of the organisms from the MLN and from feces, and detection of acid-fast bacilli in sections of the MLN—germ-free (GF) BALB/c mice exhibited clearer dose-effect relations than the flora-bearing (FB) counterparts. After intragastric administration, the organisms were probably trapped in the Peyer's patch and then transferred to the MLN at an early period (by 4–7 days), persistent infection thus being established in the MLN. Systemic involvement evolved both in athymic and euthymic mice after a prolonged period of time (more than 40 weeks) showing far more severe involvement in the former regardless of the presence of floral organisms.—KEY WORDS: alimentary tract infection, germ-free mouse, *Mycobacterium avium,* nude mouse.

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*Mycobacterium avium-intracellulare* complex is an organism distributed worldwide in the natural environment and causes diseases both in animals and man. In swine and various avian species, known to be susceptible to the organisms [6], the main natural route of infection is considered to be *via* the alimentary tract [5, 8]. Intestinal infection has also been noticed in humans [3], especially in those suffering from acquired immunodeficiency disease syndrome [25]. Regarding mammal intestinal infections due to the organism, the mode of invasion and spreading into the internal organs, and participation of immunological response in the pathogenesis have not been examined precisely, probably because a suitable animal model is lacking [12]. In the mouse, contradictory results have been reported on the susceptibility in the per oral infection (reviewed in 6). Recently, however, an outbreak of *M. avium* infection was reported in a laboratory mouse colony [21]. This suggests that the mouse might be a useful model animal for the investigation using natural routes of infection rather than parenteral infection [4, 17, 19]. Our present report describes infection through the alimentary tract in mice.

## MATERIALS AND METHODS

*Mice:* Female C57BL/6 and BALB/c mice were purchased from a commercial breeder (Japan Charles River, Kanagawa) at 4 weeks of age and used for experiment at 6 weeks of age. BALB/c mice with *nu* gene homozygously (*nu/nu*) and heterozygously (*nu/+*) were raised in this laboratory either under a germ-free (GF) or a barrier-sustained, flora-bearing (FB) condition; both sexes of these mice were used for experiment at 4 months of age. Purchased mice and FB mice (4 to 7 mice per cage) were kept on commercial pellet (F2, Funabashi Co., Ltd., Chiba) and chlorinated water. In one experiment, infected mice were kept individually. After infection, a

cage with a filter cap was placed in an isolating box equipped with a ventilation facility by negative pressure where inlet air was filtered through a coarse filter, and Hepa filter was placed at the outlet to avoid laboratory infection. Cages were changed after infection and then every two to three days. GF mice were maintained in an isolator and given autoclaved pellet (FW-1, Funabashi Co., Ltd., Chiba) and water. Periodical checking for detection of aerobic and anaerobic microbes using the heart infusion broth (BBL) and thioglycolate broth (BBL, Difco) as well as inspection of direct smear from feces showed that no contamination had occurred throughout the experiment. Wood shavings were used for bedding and all the equipment for housing mice were autoclaved before use. Bedding was changed on days 1 and 3 after infection and then twice a week thereafter.

*Mycobacterium: M. avium* strain Flamingo kept in this laboratory was used. Organisms grown on Ogawa's egg medium (Eiken Kagaku, Tokyo) were kept at −70°C after 2 passages in the chicken and selection of a transparent colony using 7H10 agar [16]. Organisms were then cultured on Ogawa's medium for 12 days at 37°C, and bacillary suspension was prepared in phosphate-buffered saline (PBS, pH 7.2) containing 0.01% Tween 80 (Tween-saline). After filtration through a glass filter, appropriately diluted suspension with the diluent (Tween-saline) was used for inoculation. A portion of the dilution was plated on Ogawa's medium to measure viable count of the inoculum.

*Administration:* Intragastric (i.g.) intubation was performed using a catheter equipped with a 1 m*l* tuberculin syringe. A volume of 0.2 m*l* per mouse was administered at a time.

*Recovery of M. avium:* In most experiments, isolation of *M. avium* was made from the caudal half of the MLN and from feces. A lymph node suspension was prepared with 0.2 m*l* PBS using a glass homogenizer, and whole sample fluid was inoculated onto one or two Ogawa's slants. Culture from feces was made principally according to Kubica [9] with a modification as follows: fecal matter (about 20 mg) was put into a small test tube and 0.2 m*l* of 2% NaOH solution was added; these were made into a suspension by using a glass bar. The fecal suspension was incubated at 37°C for 30 min. After that, 0.02 m*l* of 7.5% $H_2SO_4$ solution was added and mixed. The fluid portion of the suspension containing grossly visible particles was inoculated onto Ogawa's medium. These inoculated slants were incubated at 37°C for 4 weeks.

*Histology and measurment of affected areas on sections:* The anterior half of the MLN was usually sampled and fixed in buffered saline. Paraffin sections were stained routinely with hematoxylin-eosin and the Ziehl-Neelsen method. For complete autopsy, most of the main organs including several portions from the small and large intestines were similarly sampled and treated. Percentages of areas of lesion were measured as follows: Ziehl-Neelsen-stained preparations were projected on white paper by a Brio projector (Chiyoda Kogaku Co., Ltd.) and the margin of the affected lesion was marked. Then, the areas of lesion were cut out and the pieces of the paper were weighed.

*Recovery of organisms during an early stage after administration:* Mice were anesthetized with chloroform after which a sample of feces was taken. Then, the cervical vein was cut and the thoracic cavity was opened. A 10 m*l* volume of saline was injected into the heart. After exsanguination the abdomen was opened and the MLN was excised. Ileocecal and cecocolical regions were clipped with a nipper, and the intestine was separated into three—the small intestine, the cecum, and the colon-

rectum. The intestinal contents of the small intestine and colon-rectum were each washed out with 5 m*l* of saline by cannulating a tube equipped with a syringe while the cecum content was taken out by cutting the wall. Then, all the intestines were opened by cutting along the wall and the intestinal walls were washed by vigorous shaking using 3 changes of 20 m*l* of saline each time. The opened intestine was placed on a filter paper to remove excess saline, and grossly visible Peyer's patches (P.P.) were clipped off and pooled in groups: 3 each from duodenum-jejunum and from ileum, one from cecum, and 2–3 from colon-rectum regions. A 0.2 m*l* volume of 2% NaOH was added to fecal, MLN, and P.P. samples and the suspension was prepared in a tube or a glass homogenizer. The rest of the intestinal wall was weighed and homogenized in 10-fold volumes of 2% NaOH solution using a glass homogenizer. Samples of intestinal content were centrifuged at 2000×g for 20 min and 0.2 m*l* of 2% NaOH was added to the sediment. All the samples treated with 2% NaOH for 30 min at 37°C were neutralized with diluted $H_2SO_4$ solution and a 0.1 m*l* volume of each sample was inoculated onto Ogawa's egg medium. After incubation at 37°C for 4 weeks, the numbers of colonies developed were counted.

*Footpad reaction:* PPD prepared from *M. avium* (PPD-av) was a gift of Dr. Yugi at the National Institute of Animal Health. After 125 $\mu$g/m*l* in saline solution was prepared, 0.04 m*l* was injected into a footpad intradermally while PBS was injected another footpad. Dorsoventral thickness was measured with a dial gage caliper (Kröpplin, West Germany) at 3, 24, and 48 hr after injection. The difference of the foot thickness was expressed as swelling. Re-test reaction was made 7 days later by injecting PPD-av into the same footpad as in the previous testing and measuring footpad swelling at 3, 6, and 24 hr later as described previously [18].

## RESULTS

*Determination of criteria for detecting infection:* Since there was no available report on an adequate method to detect intestinal mycobacterial infection in mice, 20 B6 mice were given i.g. $2\times10^8$ organisms and the subsequent excretion of the organisms into feces was followed, as was the development of the footpad reaction with PPD-av by periodical testings. Organisms could be detected from feces excreted in bedding and newly voided feces sequentially in some of the mice (Table 1). Delayed footpad reaction to PPD-av was detectable by the re-testing at 6 wk and ordinary reaction at 8 wk (data not shown). Twenty mice (ten each time) were autopsied at 10 and 45 wk, and isolation of organisms from the MLN and rectal feces was performed, and the MLN and other organs were examined histopathologically. The anterior half of the MLN served for pathology and the posterior half for bacillary isolation (See Materials and Methods; Table 1). Bacilli were isolated from 18 of the 20 mice. Histopathological examination revealed lesions with acid-fast bacilli in the MLN of 17 of the 20 mice. Splenic involvement was seen in rare instances. We decided to use 3 criteria: isolation of organisms from MLN and from feces, and histologically confirmed acid-fast bacilli in MLN for detection of infection.

*Susceptibility of B6 and BALB/c strains, and observation on mice kept individually:* Batches of both strains of mice were divided into groups of 5 mice per cage and inoculated i.g. with 0.2 m*l* of serially diluted bacillary suspension. Ten weeks later, animals were sacrificed. Based on the above-described criteria, no marked difference was observed in the rate of infection between the two strains (data not shown).

Table 1. Detection of *M. avium* infection after i.g. administration

| Mouse No. | Excretion into feces (wks after infection) | | | | | | | Isolation from MLN | Pathology of MLN |
|---|---|---|---|---|---|---|---|---|---|
| | 6 | 10 | 15 | 20 | 25 | 35 | 45 | 10 or 45 wk | 10 or 45 wk |
| 1 | ND[a] | 30[a] | | | | | | 200 | –[b] |
| 2 | ′ | 6 | | | | | | 500 | + |
| 3 | ′ | 5 | | | | | | 0 | + |
| 4 | ′ | 0 | | | | | | 0 | – |
| 5 | ′ | 1 | | | | | | 200 | + |
| 6 | ′ | c[a] | | | | | | 15 | + |
| 7 | ′ | 0 | | | | | | 0 | + |
| 8 | ′ | 0 | | | | | | 0 | + |
| 9 | ′ | 0 | | | | | | 60 | + |
| 10 | ′ | 0 | | | | | | 500<br>500 | + |
| 11 | +[a] | 40 | 300 | 200 | 200 | 1 | 30 | 200 | – |
| 12 | + | c | 2 | 7 | 0 | 0 | 8 | 500 | + |
| 13 | + | 0 | 2 | c | 0 | 0 | 0 | 500 | + |
| 14 | + | 0 | c | 0 | 0 | 5 | 0 | 500 | + |
| 15 | + | 0 | 1 | 6 | 3 | 0 | ND | 30 | + |
| 16 | ND | 0 | 5 | 0 | 1 | 0 | 0 | 4 | + |
| 17 | ′ | 0 | 100 | 200 | 200 | 100 | 0 | 300 | + |
| 18 | ′ | 0 | 0 | 0 | 0 | 0 | 0 | 500 | + |
| 19 | ′ | 0 | 0 | 0 | 1 | 0 | 0 | 0 | + |
| 20 | ′ | 0 | 0 | 0 | 0 | 10 | 0 | 0 | + |

$2\times10^8$ v.u./mouse, C57BL/6. Results were expressed individually.
a) Approximate numbers of colonies. +: positive from pooled excreted feces. c: contamination. ND: not done.
b) Confirmed by the presence of acid-fast bacilli.

To establish the rationale for using positive fecal culture as evidence of infection rather than mere through-passage of bacilli which come from ingestion of feces of infected cage-mates, similar experiment was repeated on BALB/c mice kept individually. Results showed that fecal excretion of bacilli reflected the presence of infection as shown in Table 2.

*Effect of multiple administration:* To confirm whether a massive dose is necessary to produce intestinal infection, a single dose and divided doses were compared (Table 3). In both administration protocols, a total dose of more than $10^8$ infected all mice. With lesser doses, single ingestion produced a low infection rate while divided doses could not establish infection.

*Fate of bacilli during the early stages after administration:* To achieve infection, some of the ingested *M. avium* organisms must be trapped and invaded into tissue. To see when and where this process occurs, bacilli were administered to B6 mice and recovery of the bacilli was attempted at 30 min (day 0), 1, 4, and 7 days, from feces, intestinal contents, intestinal walls (deprived of P.P.), P.P., and MLN.

Results are shown in Table 4. Bacillary numbers in the intestinal contents and feces decreased rapidly from day 0 to day 7, and the bacilli could not be detected at day 7, indicating that most of the ingested bacilli were excreted rather rapidly. A similar trend was also seen in bacillary numbers in the intestinal walls and P.P. This probably

Table 2. Detection of intestinal *M. avium* infection in mice kept individually

| Mouse No. | Excretion into feces (wks after infection) | | | | | Isolation from MLN | Pathology of MLN |
|---|---|---|---|---|---|---|---|
| | 2 | 6 | 10 | 12 | 14 | 14 wk | 14 wk |
| 1 | 0 | 10[a)] | 1 | 6 | 0 | 70 | +[a)] |
| 2 | 0 | c | 0 | c | 0 | 1000 | + |
| 3 | 0 | 0 | 2 | 8 | 0 | 600 | + |
| 4 | 0 | 0 | 0 | 0 | 0 | 100 | − |
| 5 | 0 | c | 3 | 1 | 0 | 200 | + |
| 6 | 0 | 0 | 0 | 0 | 0 | 250 | + |

$2\times10^8$/mouse, BALB/c.
a) See Table 1 footnotes.

Table 3. Effect of single or divided doses to establish infection

| Experiment (Total dose) | Single injection[a)] | | | | Multiple injections[a)] | | | |
|---|---|---|---|---|---|---|---|---|
| | Isolation | | Pathology | Total incidence | Isolation | | Pathology | Total incidence |
| | MLN | Feces | MLN | | MLN | Feces | MLN | |
| A ($5\times10^8$) | 100[b)] | 25 | +[b)] | 4/4 | 500 | 70 | + | 6/6 |
| | 500 | 30 | + | | 100 | 70 | + | |
| | 200 | 50 | + | | 2 | 15 | + | |
| | 100 | 0 | + | | 70 | 25 | + | |
| | | | | | 65 | 25 | − | |
| | | | | | 100 | 25 | + | |
| B ($10^8$) | 500 | 100 | + | 5/5 | 150 | 9 | + | 6/6 |
| | 200 | c | + | | 0 | 1 | − | |
| | 500 | 0 | − | | 500 | 0 | + | |
| | 500 | 2 | + | | 200 | 100 | − | |
| | 300 | 0 | − | | 500 | 0 | − | |
| | | | | | 500 | 5 | − | |
| C ($2\times10^7$) | 0 | 0 | − | 2/5 | 0 | 0 | − | 0/8 |
| | 70 | 0 | − | | 0 | 0 | − | |
| | 0 | 0 | − | | 0 | 0 | − | |
| | 0 | 10 | − | | 0 | 0 | − | |
| | 0 | 0 | − | | 0 | 0 | − | |
| | | | | | 0 | 0 | − | |
| | | | | | 0 | 0 | − | |
| | | | | | 0 | 0 | − | |
| D ($10^7$) | 10 | 0 | + | 2/5 | | | | |
| | 0 | 0 | − | | | | | |
| | 0 | 3 | − | | | | | |
| | 0 | 0 | − | | | | | |
| | 0 | 0 | − | | | | | |

C57BL/6 mice; 8 wks after administration.
a) Total dose was injected at a time (single) or at 3- to 4-day intervals with 1/10 dose for 10 times (multiple). 0.2 m*l* of inoculum was given i.g. per mouse at each injection.
b) See Table 1 footnotes.

Table 4. Early distribution of *M. avium* after i.g. administration

| Days after administration | Intestinal contents | | | | Intestinal wall | | | | Peyer's patches | | | | Mesenteric LN. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | small int. | cecum | colon | feces | jejunum | ileum | cecum | colon | jejunum | ileum | cecum | colon | jejunum | ileocec. |
| 0 | ++[a)] | +++ | +++ | +++ | 100 | 50 | ++ | ++ | 2 | 25 | ++ | + | 0 | 0 |
| | ++ | +++ | +++ | +++ | 50 | 50 | ++ | 100 | 40 | 50 | ++ | + | 0 | 0 |
| 1 | 50 | ++ | ++ | ++ | 0 | 1 | 50 | 40 | 0 | 0 | 30 | 20 | 0 | 0 |
| | 0 | +++ | ++ | ++ | 0 | 1 | 70 | 10 | 0 | 0 | 100 | 70 | 0 | 0 |
| 4 | 100 | 50 | 100 | 2 | 5 | 15 | 7 | 0 | 10 | 0 | c | 0 | 1 | 0 |
| | 12 | 20 | 25 | 4 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 0 |
| 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | | 0 | 0 | 0 | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 1 | 20 | 7 |

$1.5\times10^8$ v.u./mouse, C57BL/6. Two mice were served in each determination.

a) Number of colonies or +, many (>100); ++, numerous (>500); +++, confluent (>1000). Less than 100 colonies were expressed by approximate numbers of colonies developed per slant.

reflects the remnants of bacilli in intestinal contents. However, positive culture from P.P.of the small intestines at day 4 and of the colon at day 7 might indicate the trapping of bacilli, although contamination from intestinal content cannot definitely be ruled out. On days 4 and 7, bacilli could be recovered from MLN although the number of bacilli detected was very small.

*Effect of flora on establishment of intestinal infection:* To see whether floral organisms intervene in the establishment of infection, GF and FB mice (BALB/c background) were infected with varied low doses. Mice were sacrificed 6 weeks after the bacillary administration. As shown in Table 5, infection in GF mice was established dose-dependently while the dose-effect response was not seen in FB mice, in which infection occurred quite inconsistently with these small doses.

*Systemic infection after a prolonged period of time:* We asked whether intestinal infection which seemed confined to the MLN can develop into systemic infection after a prolonged period of time. In addition, to determine whether immunocompetency might play a role in this localized infection, immunodeficient *nu/nu* mice of BALB/c background were also used. GF and FB mice of both *nu/nu* and *nu/+* were infected with a massive dose ($3\times10^8$) of a single ingestion. At 8 weeks, some of them were killed and confirmed to be infected. The rest were left alive and their fate was observed up to 50 weeks. Two out of 3 GF *nu/nu* mice died at 45 and 48 weeks and one of 4 FB *nu/nu* mice died at 49 weeks. These dead mice showed enlarged spleen and liver as well as MLN. Smear samples from their spleen revealed numerous acid-fast bacilli. The remaining mice were killed at 50 weeks and bacillary isolation from MLN and histopathology of the main organs were made. Numerous bacilli were isolated in all mice (data not shown). Histopathological examination revealed that systemic involvement occurred in all groups but severity of the involvement differed markedly according to immunocompetency of the mice. *nu/nu* mice of both GF and FB groups suffered from massive lesions characterized by an accumulation of macrophages engorged with acid fast-bacilli in the liver,

Table 5. Intestinal mycobacterial infection in GF and FB mice

| Dose (v.u./mouse) | GF | | | | FB | | | |
|---|---|---|---|---|---|---|---|---|
| | Isolation | | Pathology | Total incidence | Isolation | | Pathology | Total incidence |
| | MLN | Feces | MLN | | MLN | Feces | MLN | |
| $3.6\times10^7$ | 0[a)] | 0 | +[a)] | | 0 | 0 | − | |
| | 0 | 0 | + | | 0 | 0 | − | |
| | 0 | 0 | − | | 0 | 0 | − | |
| | 1000 | 0 | − | (5/7) | 0 | 0 | − | (0/7) |
| | 30 | 0 | − | | 0 | 0 | − | |
| | 30 | 0 | − | | 0 | 0 | − | |
| | 0 | 0 | − | | 0 | 0 | − | |
| $1.2\times10^7$ | 0 | 0 | − | | 0 | 0 | − | |
| | 25 | 0 | − | | 0 | 0 | − | |
| | 0 | 0 | − | (2/7) | 0 | 0 | − | (1/7) |
| | 0 | 0 | − | | 0 | 0 | − | |
| | 0 | 0 | − | | 0 | 2 | − | |
| | 50 | 50 | − | | 0 | 0 | − | |
| | 0 | 0 | − | | 0 | 0 | − | |
| $3.6\times10^6$ | 0 | 0 | − | | 0 | 2 | − | |
| | 0 | 0 | − | | 0 | 5 | − | |
| | 0 | 0 | − | (2/7) | 0 | 0 | − | (3/7) |
| | 0 | 0 | − | | 0 | 15 | − | |
| | 0 | 0 | − | | 0 | 0 | − | |
| | 0 | 20 | − | | 0 | 0 | − | |
| | 0 | 1 | − | | 0 | 0 | | |
| $1.2\times10^6$ | 0 | 0 | − | | 0 | 0 | − | |
| | 0 | 0 | − | | 0 | 0 | − | |
| | 0 | 50 | − | (1/7) | 0 | 6 | − | (2/6) |
| | 0 | 0 | − | | 0 | 6 | − | |
| | 0 | 0 | − | | 0 | 0 | − | |
| | 0 | 0 | − | | 0 | 0 | − | |
| | 0 | 0 | − | | | | | |

BALB/c-background *nu*/+ mice, 6 wks after administration.
a) See Table 1 footnotes.

spleen, and various lymph nodes and adipose tissue as well as in the intestinal tract while *nu*/+ mice of GF and FB groups exhibited well circumscribed granulomatous lesions in the liver and spleen and minimum involvement in the alimentary tract. When GF and FB mice were compared as to *nu*/+ and *nu*/*nu*, GF mice exhibited slightly more extensive lesion than FB mice. Extent of lesion in the liver and spleen was shown in Table 6.

Table 6. Extent of lesion after prolonged infection[a)]

| Group | No. mice | Percentage of affected areas | |
|---|---|---|---|
| | | Liver | Spleen |
| GF *nu*/*nu* | 1[b)] | 26.52 | 71.39 |
| GF *nu*/+ | 2 | 1.46±0.99 | 15.04± 4.99 |
| FB *nu*/*nu* | 3[b)] | 18.24±6.72 | 65.88±21.27 |
| FB *nu*/+ | 4 | 0.48±0.16 | 3.46± 0.51 |

a) Examined 50 weeks after infection with $3\times10^8$/mouse.
b) 2 others in the GF group died at 45 and 48 weeks, and 1 other in the FB group at 49 weeks, all suffering from massive involvement.

## DISCUSSION

It was confirmed that intestinal infection can be established by i.g. ingestion of *M. avium* in mice of the C57BL/6 or BALB/c strains. Both are known to be a susceptible strain when infected intravenously [7, 14, 17]. To obtain a consistent result by the per oral administration, an ingestion of a massive dose ($10^8$ level) or frequent ingestions of the $10^7$ level of the bacilli was required; however, lower doses could establish infection, although inconsistently (Tables 3 and 5). Thus, it is conceivable that a prolonged contact with contaminated materials or cage-mating with infected mice could result in spreading of infection, at least in a colony of susceptible mouse strain as reported [21, 22]. Indeed, a cross-contamination occurred when noninfected mice were cage-mated with heavily infected terminal stage-mice (unpublished observation).

The experiment which pursued the early fate of ingested organisms in the intestinal tract indicated that while most of the i.g. ingested bacilli was excreted rapidly, trapping of a small number of the organisms into the P.P. occurred. Since *M. avium* do not colonize in the mouse intestinal tract even when GF mice were used (unpublished observation), initially trapped small numbers of bacilli probably initiate infection. Then, the bacilli probably invade up to the MLN. This route of entry of enteric pathogens are known in other microbial species [2, 10, 11, 13, 15, 23]. Once loaded at the MLN, the organisms seem to multiply slowly but steadily in the organ for a considerable period of time. In other words, infection seems restricted at the MLN for a long time. This situation seems analogous to the prolonged localized infection when the organisms were injected subcutaneously [4].

Because infection is restricted during the early stages, for several months after infection the evidence of infection is barely detectable by the cultural or allergic techniques. The delayed-type hypersensitivities were rather weak, and positive culture from feces was sometimes inconsistent (Tables 1 and 2). Since positive culture from the MLN and detection of acid-fast bacilli in lesions of the MLN are confirmative evidence of infection, we used these two indices plus detection of the organisms in the feces, as an indication of established infection. We used the latter criterion to detect infection while the mice were alive. Most of the mice shedding mycobacteria in their feces were later confirmed to be infected at autopsy. However, intensive histological search for lesion in the alimentary tract could not reveal the site of bacillary shedding, unlike that reported in swine [1, 5]. The route of excretion of mycobacteria in these intestinally infected mice is not known at present.

Establishment of infection through the alimentary tract seems to be affected by indigenous flora because a dose effect relation was observed in GF mice while the incidence of infection occurred irregularly in FB mice when the infecting dose was relatively low. This barrier to the establishing of infection was probably overcome by the administration of a massive dose or by multiple administrations.

As is the case of local parenteral infection [4], infection established in the MLN did not spread readily into the liver, spleen, or other sites. However, after a prolonged time, more than 40 weeks, systemic involvement became evident. This finding is similar to an earlier description wherein mice could be infected by the oral route and extensive infection could be detected after a long period of time [6]. The extent of lesion was more severe in athymic mice of both GF and FB than in either euthymic mice, indicating that thymus-dependent protective mechanisms play an important role in the prevention of systemic involvement. In both euthymic and athymic mice, those kept in a GF

state suffered from more extensive lesion than the FB counterparts. Details of the mechanisms of this slight but obvious defence activity which is seemingly related to the presence of floral organisms await further clarification, although a similar phenomenon has frequently been reported [20, 24].

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要　約

マウスの *Mycobacterium avium* 腸管感染：上田雄幹・山崎省二・山本茂貴（国立公衆衛生院衛生獣医学部）——*Mycobacterium avium* Flaming 0 株を C57BL/6（B6），BALB/c（B/c）雌6週齢，無菌（GF）ならびにフローラ保有（FB）B/c 系ヌード（nu/nu）およびヘテロ（nu/＋）マウス雌雄 4 カ月齢に経口胃内投与後，糞便，臓器からの半定量的培養，組織切片による抗酸菌の検出を行い経口感染時の菌の侵入経路，感染菌量，感染の進展について検討した．B 6 に$10^8$ 1 回または分割（$10^7$，週 2 回）投与後，6 ～ 8 週で感染が成立し，持続的または断続的に糞便中に排菌がみられ，長期間続いた．B 6 と B/c はほぼ同程度の感受性であった．nu/＋ GFは同 FB に比べ投与量と感染率の関係が明瞭であった．投与後 7 日まで糞便，腸内容，パイエル板（PP），腸間膜リンパ節（MLN）の菌の推移を調べると，菌は腸管からは急速に減少し，4 ～ 7 日に PP および MLN にごく少数検出された．全身感染の成立を nu/nu と nu/＋ の GF および FB とで病変の形成を調べると，いずれも50週では全身諸臓器に病巣が認められたが，GF，FB ともに nu/nu が著しい病変を呈した．経口投与後早期（7 日以内）に菌はおそらく PP に捕捉され，ついで MLN に持続感染するものと思われた．感染が MLN に限局する機序には胸腺が関与する免疫機構の役割が示唆された．